

*Original Article***Tumor necrosis factor alpha and high sensitivity C-reactive protein in diagnosis of exudative pleural effusion**

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**Abstract**

**BACKGROUND:** Differentiation between exudative and transudative pleural effusions is the initial step in assessment of pleural effusion. The aim of this study was to determine whether high sensitivity C-reactive protein (hsCRP) and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) are diagnostic utilities for exudative pleural effusion.

**METHODS:** This experimental study assessed 79 patients with pleural effusion who underwent diagnostic evaluations at Imam Reza hospital, Mashhad, Iran in 2009-2010. The complete biochemical analysis of pleural fluid, pleural fluid culture, and pathological examination of pleural fluid and tissue were performed. Moreover, hsCRP and TNF $\alpha$  concentrations were measured in pleural fluid samples. The data was analyzed by student's t-test and Mann-Whitney test.

**RESULTS:** According to Light's criteria, 50 patients (63.30%) had exudative effusions while 29 subjects (36.70%) had transudative effusion. The pleural fluid concentrations of hsCRP and TNF $\alpha$  were significantly higher in the exudative group than the transudative group ( $p < 0.05$ ). At a cutoff value of 5 mg/L for hsCRP, the results showed 94% sensitivity and 96.6% specificity. Regarding TNF $\alpha$ , a cutoff value of 12.9 ng/dl represented 96% sensitivity and 93% specificity.

**CONCLUSIONS:** HsCRP and TNF $\alpha$  levels may be considered as beneficial diagnostic factors for detecting exudative effusion in patients with pleural effusion.

**KEYWORDS:** Pleural Effusion, Exudative, Transudative, HsCRP, TNF $\alpha$ .

J Res Med Sci 2011; 16(11): 1405-1409

Pleural effusion is a clinically challenging difficulty in pulmonary medicine.<sup>1,2</sup> When a patient with pleural effusion is evaluated, the initial and principal step is to discriminate between an exudative effusion and a transudative effusion.<sup>1-3</sup> Currently, the gold standard method is based upon Light's Criteria in which a pleural fluid to serum protein ratio  $> 0.5$  and/or a pleural to serum lactate dehydrogenase (LDH) ratio  $> 0.6$  discriminate pleural exudates.<sup>1</sup> However, several parameters have

been suggested for this purpose.<sup>4-11</sup>

High levels of C-reactive protein (CRP) and tumor necrosis factor alpha (TNF $\alpha$ ) are seen in inflammatory conditions such as infectious diseases, malignancy and tissue injury.<sup>4,6-9</sup> Inflammatory responses result in an exudative pleural effusion.<sup>12-14</sup> The aim of this study was to evaluate the diagnostic value of quantitative levels of high sensitivity CRP (hsCRP) and TNF $\alpha$  in pleural fluid for differentiating exudates from transudates.

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## Methods

**Patients and Assessments:** We conducted an experimental study on 79 patients with pleural effusion who were admitted at Imam Reza Hospital, Mashhad, Iran in 2009-2010. All patients underwent plain and lateral decubitus chest x-ray followed by thoracentesis under sterile conditions to obtain the pleural fluid samples. Patients did not have any bleeding tendencies. The pleural fluid samples were centrifuged at 2000 g for 10 minutes. During the next step, the cell-free supernatants were aliquoted and stored at -70°C until the final assessment. Levels of protein, LDH and glucose were measured in both serum and the pleural fluid. Gram staining and aerobic and anaerobic culture were performed on the pleural fluid samples. Ziehl-Neelsen staining was performed after homogenization and samples were cultured in Lowenstein-Jensen media in order to diagnose mycobacterium tuberculosis. HsCRP analysis was conducted by an autoanalyzer using an immunoturbidimetric method. TNF $\alpha$  levels were measured with a commercially available enzyme immunoassay (ELISA) kit (Bio-source Europe S.A.).

The patients were divided into two groups of transudative and exudative pleural effusion according to Light's criteria (on the basis of protein and LDH levels in serum and the pleural fluid). The pleural effusion was classified as exudative with at least one criterion present; otherwise it was considered as transudative. The diagnosis of tuberculous pleurisy was justified by closed pleural biopsy or through thoracoscopy. Diagnostic criteria for parapneumonic effusions were positive Gram stain, positive culture, a purulent effusion or empyema, and a white blood cell count of 5000-25000 accompanied by neutrophil predominance. Malignancy was confirmed by a positive cytology or pleural biopsy. Written informed consents were initially obtained from all patients and the study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences.

**Statistical Analysis:** The data was expressed

as mean  $\pm$  standard deviation (SD) and 95% confidence interval (95% CI) for the means and proportions were calculated. Student's t-test and Mann-Whitney test were used to compare the means of the two groups. A p value  $< 0.05$  was considered as statistically significant. Statistical analyses were conducted by SPSS (version 11.5). Cutoff points were selected based on the standard receiver operator characteristic (ROC) analysis.

## Results

Based on Light's criteria, among 79 patients with mean age of  $55.1 \pm 19$ , 29 cases (36.70%) were diagnosed as transudative and 50 cases (63.30%) as exudative pleural effusions. The transudative group included 16 (55.2%) women and 13 (44.8%) men while the exudative group included 27(55.1%) and 23 (44.9%) women and men, respectively.

The mean levels of hsCRP were  $2.9 \pm 1.27$  mg/l and  $13.7 \pm 11.08$  mg/l in the transudative and the exudative groups, respectively. There was a statistically significant difference in the mean hsCRP levels between the two groups ( $p < 0.05$ ) (Table1). To determine the efficacy of pleural fluid hsCRP measurement in distinguishing between exudative and transudative effusions a cutoff value of  $> 5$  mg/l was determined by standard ROC analysis. The sensitivity of hsCRP in differentiation between exudative and transudative pleural effusions based on this optimal cutoff level was 94%. The specificity of exudative pleural effusion was 96% (95% confidence interval: 0.89-0.98) (Table 2). The mean total TNF $\alpha$  level of exudative samples was  $16.08 \pm 17.92$  ng/dl, dramatically higher in comparison with the transudative group ( $p < 0.05$ ) (Table 1).

The diagnostic performance of TNF $\alpha$  in pleural fluid is presented in Table 2. The sensitivity and specificity of TNF $\alpha$  level in differentiating between exudates and transudates at a cutoff point of 12.9 ng/dl were 96% and 93%, respectively (Table 2). Table 2 also summarizes the positive predictive and negative predictive values of hsCRP and TNF $\alpha$ .

**Table 1.** Demographic data and pleural fluid characteristics of the study population

	Exudative pleural effusion group (n = 50)	Transudative pleural effusion group (n = 29)	P value
Age	48.8 $\pm$ 19.9	65.9 $\pm$ 11.02	p < 0.05
Gender (Female/Male)	27/23	16/13	p = 0.99
Protein	4.7 $\pm$ 1.99 mg/dl	1.7 $\pm$ 0.643 mg/dl	p < 0.05
LDH	500 $\pm$ 21.7 u/l	176 $\pm$ 30.014 u/l	p < 0.05
hsCRP	13.7 $\pm$ 11.08 mg/l	2.9 $\pm$ 1.2 mg/l	p < 0.05
TNF $\alpha$	16.08 $\pm$ 17.92 ng/dl	5.62 $\pm$ 4.12 ng/dl	p < 0.05

LDH: Lactate dehydrogenase; hsCRP: High sensitivity C-reactive protein; TNF $\alpha$ : Tumor necrosis factor alpha.

## Discussion

Pleural effusion is a clinical issue in medical practice. Accurate and rapid diagnosis of exudative pleural effusion is of crucial importance. An incorrect diagnosis may increase the application of unnecessary invasive procedures and the cost. It also defers the appropriate diagnosis.<sup>2</sup> Moreover, despite the fact that Light's criteria remain the gold standard approach in differentiating exudates from transudates, several fluid markers have been introduced for diagnostic analysis.<sup>7-11</sup> The measurement of pleural fluid interleukin-6, TNF $\alpha$ , CRP, interferon-gamma, interleukin-1 and immunological cytokines has been shown to be beneficial to the diagnosis of exudative pleural effusion.<sup>9-13</sup> In this study, we evaluated the diagnostic value of hsCRP and TNF $\alpha$  in the pleural fluid for discrimination between exudates and transudates. These two acute phase response markers were considerably higher in exudates. CRP is an acute phase protein syn-

thesized by liver in response to various inflammatory stimuli.<sup>7,8</sup> TNF $\alpha$ , which is synthesized by various activated phagocytic and nonphagocytic cells, plays a key role in inflammatory processes. It is produced during a wide variety of infections, malignancy and other inflammatory conditions.<sup>11,12,14-16</sup>

Some reports concerning the levels of pleural fluid TNF- $\alpha$  and CRP are available in the literature. While pleural effusion CRP (not hsCRP) was measured in other studies,<sup>5,7,8</sup> we evaluated the level of hsCRP and obtained outstanding results. For instance, Porcel<sup>7</sup> and Porcel et al.<sup>6</sup> evaluated CRP and several markers such as myeloperoxidase, interleukin-8, matrixmetalloproteinase-2, but not hsCRP, in pleural fluid. They focused on pneumonia and tried to distinguish between complicated and uncomplicated parapneumonia. Botana et al. compared CRP levels in benign (infectious) and malignant exudative pleural effusion.<sup>8</sup> Furthermore, compared with other related studies

**Table 2.** Diagnostic performance of pleural fluid hsCRP and TNF $\alpha$  for differentiation between exudates and transudates

Pleural fluid (exudates)	Optimal cutoff point	Sensitivity (%)	Specificity (%)	PPV(%)	NPV(%)	AUC (95%CI)
hsCRP	> 5 mg/l	94%	96.6%	97.9%	90.3%	0.968 (0.89-0.98)
TNF $\alpha$	> 12.9 ng/dl	96%	93%	85.7%	98.1%	0.95 (0.88-0.98)

PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under the curve; hsCRP: High sensitivity C-reactive protein; TNF $\alpha$ : Tumor necrosis factor alpha.

(Yilmaz et al.,<sup>9</sup> Kiropoulos et al.,<sup>4</sup> Calikoglu et al.<sup>5</sup>), we achieved different sensitivity and specificity which may be due to assessment of hsCRP instead of CRP and determining a different cut-off point. Our results for TNF $\alpha$  measurement were in accordance with other studies,<sup>11,14,17,18</sup> despite several discrepancies.<sup>15,16</sup> However, These studies assessed TNF- $\alpha$  just for pleural effusions with underlying infectious etiology.

To sum it up, according to this prospective research, we report that pleural fluid hsCRP levels > 5 mg/l and TNF $\alpha$  levels > 12.9 ng/dl

are definitely valuable in differentiating exudative from transudative pleural effusion.

## Conclusion

TNF $\alpha$  and hsCRP introduce useful markers for exudative pleural effusion.

## Acknowledgement

This study was financially supported by Research Vice Chancellor, Mashhad University of Medical Sciences (Research No. 85463 and Thesis No. 2008).

## Conflict of Interests

Authors have no conflict of interests.

## Authors' Contributions

FR and HA designed and carried out the research, collected the data, interviewed the patients, coordinated the study, participated in all of the research, and prepared the manuscript. FA provided assistance in designing and conducting the research, collected the data, and participated in manuscript preparation. SMRP provided assistance in laboratory tests. HE performed the statistical analyses. SS corrected the English manuscript and revised further statistical data. All authors have read and approved the content of the manuscript.

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